Cell
Leading Edge
Previews

# Bcl-2 Proteins and Apoptosis: Choose Your Partner

Gordon C. Shore[1,*] and Mai Nguyen[1]
[1]Department of Biochemistry and Goodman Cancer Center, McGill University, Montreal QC H3G 1Y6, Canada
*Correspondence: gordon.shore@mcgill.ca
DOI 10.1016/j.cell.2008.11.029

**The Bcl-2 family protein Bax is a key effector of apoptosis. Lovell et al. (2008) now describe the reconstitution and regulation in liposomes of tBid-mediated membrane penetration and oligomerization of Bax. These and other recent studies shed new light on the control of permeabilization of the mitochondrial outer membrane during apoptosis.**

Members of the Bcl-2 family constitute the critical effectors and regulators of programmed cell death or apoptosis (Youle and Strasser, 2008). In the face of stress stimuli, whether a cell survives or undergoes apoptosis is dependent on the extent of pairing between the Bcl-2 family members that promote cell death with family members that promote cell survival. For the most part, these interactions involve the docking of the Bcl-2 homology 3 (BH3) domain of proapoptotic family members into a groove on the surface of prosurvival members. Yet, how this translates into regulation of permeabilization of the outer mitochondrial membrane, a key element of the intrinsic pathway of apoptosis, has been challenging to decipher, and a number of conflicting results remain to be reconciled (Youle and Strasser, 2008). In this issue, Lovell et al. (2008) report the reconstitution in liposomes of the series of dynamic events that lead to membrane permeabilization by the proapoptotic Bcl-2 proteins, tBid and Bax. They also provide insight into how a prosurvival Bcl-2 family member, Bcl-xL, antagonizes these events and how this antagonism is overcome by the proapoptotic member Bad.

The presence of Bcl-2 homology (BH) domains defines the membership of the Bcl-2 family, which is divided into three main groups depending upon the particular BH domains present within the protein. The prosurvival members such as Bcl-2, Bcl- xL, and Mcl-1 contain BH domains 1–4, whereas Bax and Bak the proapoptotic effectors of mitochondrial outer membrane permeabilization (MOMP) during apoptosis, contain BH domains 1–3. A larger group of proapoptotic members, including Bid, Bim, and Bad, among others, contain only the BH3 domain and serve to couple upstream stress stimuli to downstream modulation of the multi-BH domain members.

There are two important factors that have contributed to the challenge of understanding how interactions between Bcl-2 family members regulate membrane permeabilization. First, the membrane appears to play an active role in modulating Bcl-2 family protein structure, interactions, and function. Second, stress stimuli can lead to dynamic changes in protein conformation, culminating in the penetration of otherwise cytosolic domains into the lipid bilayer, where the relevant protein-protein interactions take place (Annis et al., 2005; Dlugosz et al., 2006; Billen et al. 2008). This makes it particularly difficult to elucidate valid interactions between the Bcl-2 members with cell-based assays in which membranes are destroyed.

In unstressed cells, Bax resides inert in the cytosol, probably because of the fact that its C-terminal membrane anchor is sequestered within the hydrophobic surface groove (Suzuki et al., 2000). Changes at the extreme N terminus, on the other hand, can derepress the sequestered membrane anchor, resulting in insertion of the protein into its target membranes, the mitochondrial outer membrane, and the endoplasmic reticulum (Goping et al., 1998). At the membrane, Bax undergoes a dramatic conformational change caused by the insertion of helices 5 and 6 into the bilayer (Annis et al., 2005), and it is this conformer that oligomerizes. Bax then assumes a structure reminiscent of the pore-forming colicins and diptheria toxins. The bilayer, therefore, is not simply a barrier that Bax can breach but is an active participant in the initial activation of Bax (and likely explains why functional reconstitution in liposomes is possible).

What initiates the physiological activation of Bax? One model suggests that the active forms of certain BH3-only proteins (tBid, Bim, and potentially Puma) interact with Bax, seeding the cascade of Bax transformations (Youle and Strasser, 2008; Letai et al., 2002). However, what has been missing until now is the geographic and kinetic elucidation of this interaction. Employing an amphiphilic BH3 peptide derived from Bim, which is locked into its functional conformation, Gavathiotis et al. (2008) have recently found a noncanonical BH3 binding site located near the N terminus of Bax. It is on the opposite face of the protein from the canonical BH3 binding site of the antiapoptotic members. It is attractive to speculate that this binding provides the physiological stimulus to cause the N-terminal changes that induce derepression of the membrane anchor, leading to membrane insertion and activation of Bax. Other non-BH3 stimuli such as heat and pH might mimic these Bim-induced changes at the N terminus.

The liposome reconstitution experiments of Lovell et al. (2008), presented in this issue, add important further support for this model. The authors employed fluorescence resonance energy transfer

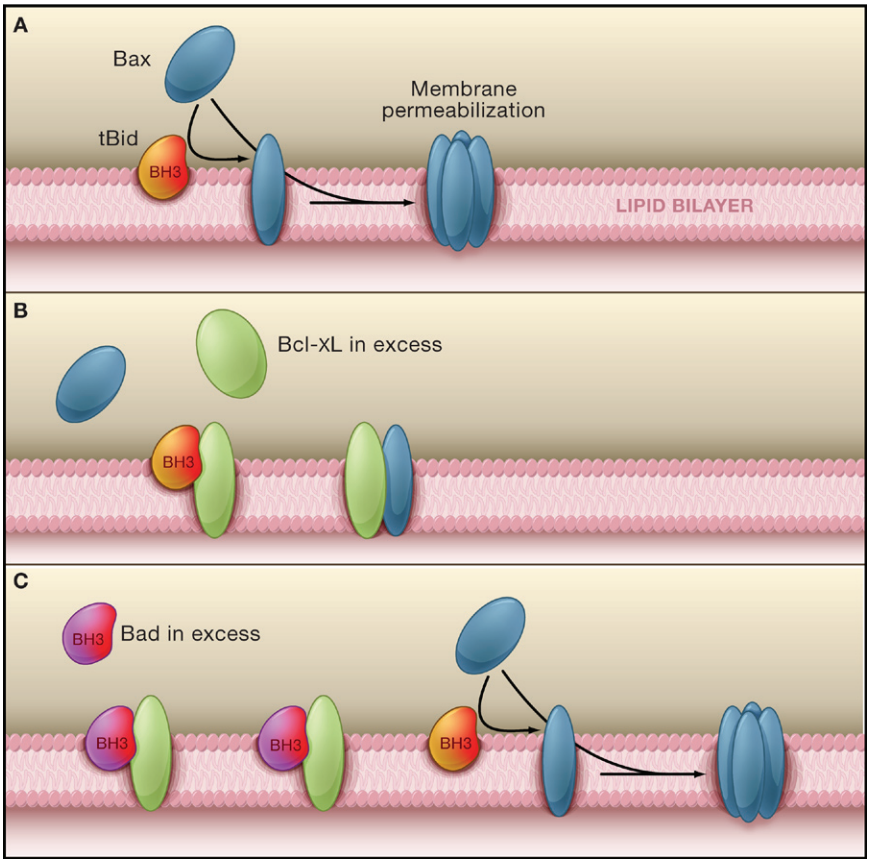


**Figure 1. Regulation of Membrane Permeabilization by Bcl-2 Family Proteins**

(A) Cleavage by caspase-8 generates tBid (orange), which rapidly migrates to the membrane (Lovell et al., 2008), where its BH3 domain (red) seeds the insertion of Bax (blue) into the membrane via binding to a non-canonical BH3 binding site near the N terminus of Bax (Gavathiotis et al., 2008). The resulting activated Bax molecules can now oligomerize both between themselves and by recruiting inactive Bax conformers from the cytosol, resulting in membrane permeabilization (Dlugosz et al., 2006, Billen et al., 2008).

(B) Bcl-xL (green) in excess competes with Bax to interact with tBid, but, unlike the binding of the tBid BH3 domain to the N terminus of Bax, the canonical interaction of tBid with the BH3 binding groove of Bcl-xL is relatively stable. Similarly, Bcl-xL can interact with the exposed BH3 of the activated conformer of Bax, forming nonproductive dimers that cannot oligomerize. Thus Bcl-xL has at least two ways of inhibiting Bax oligomerization (Dlugosz et al., 2006; Billen et al., 2008; Lovell et al., 2008).

(C) A "sensitizing" BH3-only protein Bad (purple) can displace tBid or activated Bax from sequestration by Bcl-xL (Billen et al., 2008; Letai et al., 2002; Lovell et al., 2008), freeing tBid to seed the activation of additional Bax molecules, leading to Bax-mediated membrane permeabilization.

(FRET) to measure, both in real time and simultaneously in a single reaction, protein movement, interactions between binding partners, and penetration of Bcl-2 family proteins into the bilayer. They find that tBid migrates and inserts into the liposome very quickly and is required for Bax activation. Consequently, it initiates Bax activation at the membrane, coupling this activation to the slower kinetics of membrane insertion of Bax into the bilayer. Thus, tBid appears to act like an essential membrane receptor for membrane permeabilization mediated by Bax (Figure 1), that is, at least in this simplified system free of influences that might otherwise occur in an intact cell.

The studies of Lovell et al. also address the current controversies around the mechanisms of antagonism of Bax-mediated membrane permeabilization by prosurvival Bcl-2 proteins. The prosurvival proteins Bcl-2 and Bcl-xL, at least in reconstituted membranes in vitro, exhibit remarkable similarities to Bax in almost all respects, including the ability to undergo conformational alterations and penetration into the bilayer of helices 5 and 6 (Dlugosz et al., 2006; Billen et al., 2008). An important exception is that dimers between activated conformers of Bcl-2 or Bcl-xL and Bax, in contrast to activated Bax itself, cannot further oligomerize within the bilayer and therefore represents a dead end interaction (Billen et al., 2008) (Figure 1). In theory, Bcl-2/Bcl-xL antagonism of Bax could operate at multiple steps in the Bax activation pathway, although certain models put forth mutually exclusive explanations (Youle and Strasser, 2008; Letai et al., 2002; Willis et al., 2007). One model favors sequestration by Bcl-2/Bcl-xL of BH3-only proteins that directly activate Bax, with other "sensitizing" BH3-only proteins (such as Bad, which does not interact with Bax) having the capacity to displace the sequestered activator BH3, freeing it to go on to activate Bax (Letai et al., 2002). Another model favors the possibility that the antagonism of Bax stems exclusively from the constitutive interaction of Bax with Bcl-2 or Bcl-xL. Displacement of Bax by a BH3-only protein initiates the Bax activation cascade by an as yet undetermined mechanism (Willis et al., 2007). Based on the new evidence in favor of activating BH3-only proteins, elements of the latter model may kick in downstream of Bax activation (by BH3-dependent or -independent means) (Figure 1). The direct model, if correct, assumes that Bcl-2 and Bcl-xL represent a massive sink for interaction with activator BH3-only proteins relative to binding to Bax. This implies a significantly higher binding affinity to activator BH3-only proteins, a requirement for a significant excess of Bcl-2 and Bcl-xL relative to Bax, and/or kinetics that favor the differential appearance of competing binding partners at the membrane surface.

The FRET analyses of Lovell et al. (2008) support the notion that Bcl-xL can effectively sequester almost the entire population of tBid molecules, with a smaller amount of membrane-integrated Bcl-xL/Bax dimers also detected. Bad in excess over tBid (10×) displaced tBid, which subsequently activated Bax (Figure 1). It should be emphasized, however, that both the order of addition of Bcl-xL to liposomes (which preceded tBid) and the relatively modest excess of Bcl-xL relative to tBid (2×) likely influenced the preferred choice of binding partners in this system. It would be intriguing to systematically analyze this.

Notwithstanding the numerous secondary elements of control over Bcl-2 family protein-protein interactions that exist within an intact cell (Youle and

Strasser, 2008), the liposome reconstitution system developed by Lovell et al. (2008) represents a powerful biochemical means to investigate the dynamic behavior of Bcl-2 proteins in intact membranes. In the cell, multiple members of the three Bcl-2 subgroups are typically expressed. Unlike Bcl-xL and Bax, several of these (Bcl-2, Mcl-1, and Bak) are constitutively tethered to the mitochondrial outer membrane via their transmembrane segments prior to activation. The similarities and differences of the interactions between different Bcl-2 binding partners at the membrane, and their influence on membrane permeabilization, can now be explored.

## REFERENCES


Annis, M.G., Soucie, E.L., Dlugosz, P.J., Cruz-Aguado, J.A., Penn, L.Z., Leber, B., and Andrews, D.W. (2005). EMBO J. *24*, 2096–2103.

Billen, L.P., Kokoski, C.L., Lovell, J.F., Leber, B., and Andrews, D.W. (2008). PLoS Biol. *6*, 1268–1280.

Dlugosz, P.J., Billen, L.P., Annis, M.G., Zhu, W., Zhang, Z., Lin, J., Leber, B., and Andrews, D.W. (2006). EMBO J. *25*, 2287–2296.

Gavathiotis, E., Suzuki, M., Davis, M.L., Pitter, K., Bird, G.H., Katz, S.G., Tu, H.C., Kim, H., Cheng, E.H., Tjandra, N., and Walensky, L.D. (2008). Nature *455*, 1076–1081.

Goping, I.S., Gross, A., Lavoie, J.N., Nguyen, M., Jemmerson, R., Roth, K., Korsmeyer, S.J., and Shore, G.C. (1998). J. Cell Biol. *143*, 207–215.

Letai, A., Bassik, M.C., Walensky, L.D., Sorcinelli, M.D., Weiler, S., and Korsmeyer, S.J. (2002). Cancer Cell *2*, 183–192.

Lovell, J.F., Billen, L.P., Blinder, S., Shamas-Din, A., Fradin, C., Leber, B., and Andrews, D.W. (2008). Cell, this issue.

Suzuki, M., Youle, R.J., and Tjandra, N. (2000). Cell *103*, 645–654.

Willis, S.N., Fletcher, J.I., Kaufmann, T., van Delft, M.F., Chen, L., Czabotar, P.E., Ierino, H., Lee, E.F., Fairlie, W.D., Bouillet, P., et al. (2007). Science *315*, 856–859.

Youle, R.J., and Strasser, A. (2008). Nat. Rev. Mol. Cell Biol. *9*, 47–59.


# Divided within: Heterogeneity within Adult Stem Cell Pools


**Marc H.G.P. Raaijmakers[1,2,3] and David T. Scadden[1,2,3,*]**

[1]Center for Regenerative Medicine and Cancer Center, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114, USA
[2]Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA 02138, USA
[3]Harvard Stem Cell Institute, Cambridge, MA 02138, USA
*Correspondence: dscadden@mgh.harvard.edu

DOI 10.1016/j.cell.2008.11.034


**In this issue, Wilson et al. (2008) demonstrate that there are two functional subsets of hematopoietic stem cells that have distinctive kinetics of cell cycling. They present evidence that cells may transition between the two kinetic states, establishing one subpopulation that is ready to proliferate and another that is a deeply quiescent reserve.**


The study of hematopoiesis has been remarkably fruitful for uncovering basic principles of tissue organization including lineage hierarchy and in understanding how organismal outcomes are affected by the responses of specific cell types. An example is host defense, where the blood is remarkably nimble at shifting its production of cells into high gear when challenged with infection or bleeding. Although hematopoietic stem cells (HSCs) ultimately make this response possible, the majority of cell production occurs in a more mature population, the progenitor cells. However, these cells have little capacity for self-renewal and are therefore rightly designated transient amplifying cells. Consequently, the stem cell must be feeding cells into the self-depleting progenitor pool on an ongoing basis, which would require some degree of HSC proliferation. Yet, if the stem cell pool is mitotically active, even if at a rate far below that of the transient amplifying pool, the cells would likely be susceptible to genotoxic injury and subsequent apoptosis. If so, HSCs might fail to preserve the regenerative pool necessary to overcome a hematopoietic crisis created by prolonged genotoxic stress. It has been unclear how a single population of stem cells reconciles these two competing duties, that is, supplying transit-amplifying cells while maintaining a deep reserve of stem cells for long-term repopulation. As a potential resolution to this conundrum, new findings by Wilson et al. (2008) suggest that stem cells, even within a given tissue, may represent a spectrum of cells with heterogeneous capabilities.

Although murine HSCs do have a lower rate of cell cycling compared to progenitor pools, it has been shown that their cell cycling frequency is surprisingly rapid, with 6%–8% of HSCs entering cycle daily (Cheshier et al., 1999; Kiel et al., 2007). In addition, recent efforts have defined mammalian intestinal and hair follicle cell pools with convincing stem cell features that are actively cycling (Barker et al., 2007; Jaks et al., 2007). Small intestinal stem cells are estimated to turn over daily, for example (Barker et al., 2007).